# The Quantitative Genetics of Maximal and Basal Rates of Oxygen Consumption in Mice

**Michael R. Dohm,[1] Jack P. Hayes[2] and Theodore Garland, Jr.**

*Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706*

Manuscript received August 23, 2000
Accepted for publication June 8, 2001

ABSTRACT

A positive genetic correlation between basal metabolic rate (BMR) and maximal ($\dot{V}O_2$max) rate of oxygen consumption is a key assumption of the aerobic capacity model for the evolution of endothermy. We estimated the genetic ($V_A$, additive, and $V_D$, dominance), prenatal ($V_N$), and postnatal common environmental ($V_C$) contributions to individual differences in metabolic rates and body mass for a genetically heterogeneous laboratory strain of house mice (*Mus domesticus*). Our breeding design did not allow the simultaneous estimation of $V_D$ and $V_N$. Regardless of whether $V_D$ or $V_N$ was assumed, estimates of $V_A$ were negative under the full models. Hence, we fitted reduced models (*e.g.*, $V_A + V_N + V_E$ or $V_A + V_E$) and obtained new variance estimates. For reduced models, narrow-sense heritability ($h^2_N$) for BMR was $<0.1$, but estimates of $h^2_N$ for $\dot{V}O_2$max were higher. When estimated with the $V_A + V_E$ model, the additive genetic covariance between $\dot{V}O_2$max and BMR was positive and statistically different from zero. This result offers tentative support for the aerobic capacity model for the evolution of vertebrate energetics. However, constraints imposed on the genetic model may cause our estimates of additive variance and covariance to be biased, so our results should be interpreted with caution and tested via selection experiments.

MAXIMAL and minimal rates of aerobic metabolism are commonly studied traits in comparative and evolutionary physiology (Blaxter 1989; Garland and Adolph 1991; Feder and Burggren 1992; Garland and Carter 1994). Measures of minimal, or resting, metabolic rates (also termed basal, BMR, or standard, SMR, depending on details of measurement conditions) are often used to infer the minimum maintenance requirements of an organism (Blaxter 1989). Maximal rates of oxygen consumption during forced exercise ($\dot{V}O_2$max) indicate upper bounds on the intensity of activity that animals can sustain by aerobic metabolism (Astrand and Rodahl 1986; Wagner 1996; Weibel *et al.* 1998). Both traits are thought to be of selective importance (Bennett and Ruben 1979; Chappell and Snyder 1984; Lynch 1992, 1994; Hayes and O'Connor 1999). Lower values of BMR are presumed to be advantageous because maintenance costs will be lower (but *cf.* Richardson *et al.* 1994). Higher values of $\dot{V}O_2$max are presumed to be advantageous because higher levels of activity or thermoregulatory function can be supported aerobically (Chappell and Snyder 1984; Hayes 1989).

Although rates of resting and maximal aerobic metabolism are determined in part by distinct organ systems—basal rates by the visceral organs and brain, maximal rates by cardiac and skeletal muscle (Astrand and Rodahl 1986)—maximal and resting rates of aerobic metabolism may be functionally linked in vertebrates. $\dot{V}O_2$max is usually 5–10 times BMR (or SMR), an empirical generalization that applies to mammals, birds, reptiles, amphibians, and fishes, over a broad range of body masses (Bennett and Ruben 1979; Schmidt-Nielsen 1984; Gatten *et al.* 1992; Hinds *et al.* 1993; Walton 1993; Dutenhoffer and Swanson 1996). Compared to reptiles and other ectotherms, mammals and birds have substantially higher aerobic capacity (*i.e.*, $\dot{V}O_2$max). They also have substantially higher resting metabolic rates. Compared with reptiles, mammals have greater lung vascularization, ventilation rates, blood $O_2$ carrying capacity, relatively larger visceral and skeletal muscle, and a variety of cellular and subcellular differences that are thought to contribute to the higher rates of metabolism (Ruben 1995).

These observations led Bennett and Ruben (1979) to develop the aerobic capacity model for the evolution of endothermy. The model proposed that, in the ancestors of mammals and birds, natural selection increased aerobic capacities to support vigorous but aerobically sustainable activity. Elevated resting metabolism was thought to evolve owing to a hypothesized link between resting metabolism and aerobic capacity. A key, implicit assumption of the model is that a positive genetic correlation between resting and maximal rates of metabolism must have been present in the ancestors of birds and mammals (Hayes and Garland 1995). If this correla-

*Corresponding author:* Theodore Garland, Jr., Department of Biology, University of California, Riverside, CA 92521.

[1] *Present address:* Department of Biology, University of Hawaii, 200 W. Kawili St., Hilo, HI 96720.

[2] *Present address:* Department of Biology, University of Nevada, Reno, NV 89557.

tion is a pervasive, ineluctable feature of the design of terrestrial vertebrates, then it may still be present in extant endotherms. Although genetic correlations are unlikely to remain unchanged over long periods of evolutionary time (Turelli 1988; Gromko 1995; Lascoux 1997), their persistence is possible for traits that are closely coupled through biomechanics, physiology, or development (Cheverud 1982).

Several studies have tested for and found weak phenotypic associations between maximal and resting aerobic metabolic rates (reviewed by Hayes and Garland 1995), but phenotypic correlations are generally inadequate for testing genetic hypotheses (reviewed by Roff 1997). Both the sign and magnitude of phenotypic and genetic correlations may differ because the phenotypic correlation is a function of genetic and environmental correlations (Falconer and Mackay 1996). The primary objective of this study was, therefore, to test for genetic covariation between BMR and $\dot{V}O_2$max in house mice. [Two measures of locomotor performance were also obtained on these mice: maximal sprint running speed and swimming endurance (Dohm *et al.* 1996).] The widespread existence of positive genetic correlations between BMR and $\dot{V}O_2$max would lend support to the aerobic capacity model of endothermy. Ours represents the first attempt to test for such a correlation in any group of animals.

## MATERIALS AND METHODS

**Strain history and animal husbandry:** We studied the outbred, genetically variable Hsd:ICR strain of house mice (*Mus domesticus*) obtained from Harlan Sprague Dawley, Inc., Indianapolis (room 202, Barrier A). Outbred laboratory strains designated Swiss Webster, including the strain we used, have levels of genetic variation similar to those of wild populations of house mice (Rice and O'Brien 1980). The ICR strain is genetically heterogeneous [polymorphic at 36.4% of 11 allozyme loci studied, average heterozygosity between 10.3% (founders) and 11.7% (offspring); Carter *et al.* 1999; see also Hauschka and Mirand 1973; Rice and O'Brien 1980]. ICR mice have been the subjects for several evolutionary physiological (*e.g.*, Hayes *et al.* 1992; Friedman *et al.* 1992; Dohm *et al.* 1994; Richardson *et al.* 1994; Garland *et al.* 1995; Swallow *et al.* 1998a,b) and quantitative genetic analyses (*e.g.*, Riska *et al.* 1984; Dohm *et al.* 1996). Details of the foundation population for the Hsd:ICR mice and other relevant information about the strain have been published previously (Dohm 1994; see also Hauschka and Mirand 1973).

**Breeding design:** Data were obtained from five measurement blocks, each block consisting of five founder males (seven in the first block) and up to 22 founder females and their offspring. Founder mice obtained from HSD were not related. We employed a nested breeding design, with cross-fostering (Newman *et al.* 1989), to allow identification of the relative magnitude of direct genetic and environmental effects on individual variation in phenotypic traits. Each male was harem mated to 4 or 5 randomly selected females. At birth, litters were standardized to eight pups per dam, and each offspring was toe-clipped for identification. We cross-fostered pups at birth between two or more dams that had given birth on the same day. Each dam raised up to four pups of her own and up to four pups from other dams. Sixty-seven families were included in the cross-fostering design. Pups were weaned at 19 days of age and four offspring per dam (two cross-fostered, two not cross-fostered) were randomly selected for physiological measurements. At weaning, male and female offspring were placed in same-sex groups of four or five per cage.

Measurements were made on founder mice (*i.e.*, breeders and nonbreeders obtained from Harlan Sprague Dawley) and on offspring from the 67 cross-fostered families. Additional husbandry details have been published elsewhere (Hayes *et al.* 1992; Dohm 1994; Dohm *et al.* 1996).

**Measurement schedule:** BMR measurements were initiated after the mice reached 30 days of age (mean ± SD = 35.4 ± 2.57, range 30–43). Food was removed at ~1800 hr (CST) the night before. Maximal oxygen consumption was measured at least 3 days, but not longer than 9 days, after BMR was measured (6.2 ± 2.15 days). Because of technical difficulties, $\dot{V}O_2$max was not determined for the founder mice of the first experimental block. Sample sizes varied for each measurement and are listed in Table 1.

**Basal metabolic rate:** BMR of postabsorptive [not digesting a meal (Hart 1971)] mice was measured once within the thermal neutral zone for house mice [32° (Hart 1971; Lacy and Lynch 1979)]. Mice were fasted overnight and placed in metabolic chambers the next morning. Metabolic chambers were connected to an open-circuit respirometry system that has been described in detail elsewhere (Hayes *et al.* 1992; Richardson *et al.* 1994). Briefly, up to seven mice were monitored simultaneously. Each mouse and a control chamber received air at 200 ml/min standard temperature and pressure dry (STPD) from upstream thermal mass flow controllers (Sierra Instruments, Inc. Monterey, CA, Side-Trak model 844). Water and $CO_2$ were removed from the excurrent air. Excurrent air from each chamber was monitored for at least 7.5 consecutive minutes of each hour (longer if fewer than seven mice were being measured) by an Applied Electrochemistry S-3A/II oxygen analyzer (Ametek, Pittsburgh) interfaced to a computer. Excurrent oxygen concentration was determined once every 5 sec (the average of two values) and $\dot{V}O_2$ was calculated using equation 4 from Hill (1972, p. 261). The lowest and second lowest 5 min of $\dot{V}O_2$ for each mouse during the 8 hr of monitoring were calculated. The lower of the two values was taken as BMR and used for all genetic analyses. We also compared the two lowest values as an index of repeatability.

**Maximal rates of oxygen consumption:** $\dot{V}O_2$max during forced exercise was measured on a motorized treadmill with an incremental step test according to a protocol used extensively by us (Friedman *et al.* 1992; Hayes *et al.* 1992; Dohm *et al.* 1994; Swallow *et al.* 1998b). A mouse was placed in a small Plexiglas chamber held just above the surface of the treadmill belt, thus allowing inflow of room air. Mice were first placed in the chamber while the treadmill was off and resting $O_2$ was recorded for 1–2 min. Mice were then induced to run by prodding with a straightened paper clip inserted through a small hole at the rear of the chamber and by a mild electric current (3–12 mA; provided by a grid of 12 2-mm-diameter bars spaced 5 mm apart). From an initial speed of 1.0 kmh, treadmill speed was increased every 2 min by 0.5 kmh, up to a maximum of 4.5 kmh. Trials were ended when $\dot{V}O_2$ failed to increase as tread speed increased and the mouse did not keep pace with the moving belt. All mice reached a speed of at least 2.0 kmh.

Air was drawn from the chamber via eight ports (each 3 mm in diameter) in its top, through columns of Drierite and Ascar-

ite II to remove water vapor and $CO_2$, respectively, and then passed through a thermal mass flow controller set at 2500 ml/min STPD. This flow rate ensured rapid chamber washout; time to initial response was <5 sec. We also determined the effective volume of the system (540 ml) and made instantaneous corrections for chamber washout (Bartholomew *et al.* 1981), because the standard equations (Equation 4a in Withers 1977, p. 122) are for use under steady-state (equilibrium) conditions. With the rapid washout of this system, the instantaneous correction was relatively minor and we elected to use the steady-state values for genetic analyses because the four-component restricted maximum likelihood (REML) models failed to converge for the instantaneous $\dot{V}O_2$ data. Oxygen concentration in the excurrent air was recorded every second (average of 20 consecutive readings) by the oxygen analyzer and computer described in the BMR section above. Oxygen consumption generally increased with increasing speed, and the highest 1-min period of oxygen consumption during a trial was taken as $\dot{V}O_2$max, consistent with previous studies (*e.g.*, Friedman *et al.* 1992; Hayes *et al.* 1992; Dohm *et al.* 1994; Swallow *et al.* 1998b).

**Data analyses:** We used multiple regression to remove possible confounding effects of body mass, age at measurement, time of day at measurement, and other relevant covariates prior to genetic analyses of the metabolic traits. We used a stepwise selection algorithm (entry level $P = 0.05$, removal level $P = 0.10$) to identify significant covariates. Measurement block, sex, and whether an individual was a founder (*i.e.*, breeder and nonbreeder mice obtained from HSD), or an offspring born in our laboratory, were scored as dummy variables, and the product of the sex-by-founder dummy variables was also used. For BMR, we also used total fasting time (defined as the time between removal of food and the midpoint of the lowest 5-min interval) as a covariate. For fasting time, age, and time at measurement, second order polynomials (*e.g.*, fasting time squared) were also used to allow for nonlinear associations with the dependent variable. (*Z*-scores for the first order terms were obtained before squaring to reduce the correlation between first and second order terms.) We also identified significant covariates for the various body mass measures recorded during the experiment. Throughout we use correlation in the standard sense of a Pearson product-moment correlation.

We estimated genetic parameters for the following residual metabolic traits (transform used): BMR (no transform); the higher of the two $\dot{V}O_2$max trials, $\dot{V}O_2$max ($\log_{10}$); and the average of the two trials, avg. exercise $\dot{V}O_2$ ($\log_{10}$). Average exercise $\dot{V}O_2$ was calculated after first subtracting the difference in mean value between the first and second trials from each second-day value, because the mean $\dot{V}O_2$ on the second day was slightly higher than the mean of trial 1 exercise $\dot{V}O_2$. This correction is necessary prior to calculation of heritability because the difference in means may inflate the within-family variance component, leading to an underestimation of heritability (Cheverud 1982). We also estimated genetic parameters for two measures of body mass (both $\log_{10}$ transformed), after accounting for covariates: body mass taken just before placing mice in the chamber for determining BMR and mean body mass measured during the $\dot{V}O_2$max trials.

**Genetic model fitting:** We used the following rules of thumb for evaluating the suitability of models. The models should not violate theoretical constraints. For example, a model that predicts dominance genetic effects in the absence of additive genetic effects is unlikely (Falconer and Mackay 1996). Furthermore, large, negative variance estimates for one or more components of the model make the model suspect. We used Shaw's (1987) REML program. Iterations were continued until the difference in successive likelihoods was <0.0001.

We used a linear model that allowed estimation of four variance components: $V_A$, additive genetic effects; $V_C$, common environmental effects; $V_E$, effects of environment unique to individuals; and either $V_N$, prenatal maternal effects, or $V_D$, dominance genetic effects (for additional details, see Dohm 1994; Dohm *et al.* 1996). Dominance genetic and prenatal effects were confounded because the breeding design did not yield a pedigree capable of the simultaneous estimation of $V_D$ and $V_N$. We therefore evaluated the fit to the data for models that yielded estimates for $V_A + V_C + V_E$ and either $V_N$ or $V_D$. This was done by changing the coefficient for full sibs from 0.25 for $V_D$ to 1.0 for $V_N$ in the REML program. Models with $V_D$ tended to yield negative variance estimates for $V_E$ (see results), whereas models with $V_N$ yielded positive, interpretable estimates for $V_E$. We assume no epistatic interaction and no contribution of genotype-by-environment interaction or correlation (*e.g.*, across measurement blocks) to the total variance.

We first used single-character (univariate) data sets to obtain parameter estimates and model-fit statistics for the full *A[ND]CE* model (*i.e.*, the model containing all four estimable variance components, $V_A + [V_N \text{ or } V_D] + V_C + V_E$) and for a series of nested submodels (*A[ND]E*, *ACE*, *AE*, *CE*, *E*), where *A* is the additive variance, *N* is the prenatal maternal effects variance, *D* is the dominance (interaction within a locus) genetic variance, *C* is the postnatal maternal and other common environmental variances (source of environmental variation that contributes to the variance between families) variance, and *E* is the environmental effects variance (Falconer and Mackay 1996).

For BMR, the full *A[ND]CE* model yielded negative estimates of additive variance and common environmental variance. For $\dot{V}O_2$max, these same components of variance were also negative. Consequently, we fitted reduced models that estimated only additive and environmental variances while constraining the dominance (or prenatal effects variance) and common environmental variances to zero. These reduced models always yielded positive variance estimates. The estimates for $V_A$ may be biased upward if substantial dominance genetic, prenatal effects, or common environmental effects variance were indeed important causal components of phenotypic variation (Lynch and Walsh 1998; see discussion).

We also estimated two-trait (bivariate) reduced models that partitioned the covariation between $\dot{V}O_2$max and BMR residuals into additive genetic and unique environmental sources of covariation. As for the univariate models, these variance estimates may be biased upward (see discussion). Phenotypic ($r_P$) and additive genetic ($r_A$) correlations between traits were calculated as: $r_X = COV_{x1,2} / (V_{x1} \cdot V_{x2})^{0.5}$, where *x* refers to the phenotypic or additive genetic effect, $COV_{x1,2}$ refers to the covariance of the *x*th type, and $V_{x1}$ and $V_{x2}$ refer to the variance for the first and second trait, respectively.

We tested the significance of the additive variances and covariances with likelihood ratio tests. For example, the likelihood of additive genetic variance (*AE* model) was compared to the likelihood of a constrained model (*E*) with the additive genetic component set to zero. Twice the difference in log-likelihoods (*LL*) is distributed approximately as a chi square ($\chi^2$) with the degrees of freedom equal to the number of parameters constrained to zero (one in this case). For example, the additive genetic covariance would be judged significant only if the goodness-of-fit measure, $\chi^2$, was larger than a specified critical value (*e.g.*, for one constrained parameter the critical $\chi^2$ for a two-tailed test is 3.841 at $P = 0.05$). In contrast, the test of the variance components is a one-tailed test and the corresponding critical $\chi^2$ at $P = 0.05$ is 2.706 (Shaw and Geyer 1997).

**TABLE 1**

**Descriptive statistics for metabolic traits and body mass measured on outbred, genetically variable laboratory strain of house mice**

| Traits | | *N* | Mean | SD | Min | Max |
|---|---|---|---|---|---|---|
| Basal metabolic rate (ml $O_2$/hr)[a] | Female founder[b] | 96 | 38.2 | 6.31 | 12.5 | 55.5 |
| | Male founder | 29 | 47.9 | 8.27 | 32.6 | 68.2 |
| | Female offspring | 122 | 35.5 | 7.12 | 13.9 | 57.0 |
| | Male offspring | 124 | 43.0 | 11.06 | 11.8 | 77.0 |
| Body mass (g) at start of BMR trial[c] | Female founder | 96 | 21.63 | 2.234 | 16.92 | 28.11 |
| | Male founder | 29 | 25.92 | 2.668 | 21.07 | 30.54 |
| | Female offspring | 122 | 19.33 | 2.109 | 14.84 | 23.96 |
| | Male offspring | 123 | 22.85 | 3.064 | 14.91 | 30.07 |
| Avg. exercise $\dot{V}O_2$ (ml $O_2$/hr)[d] | Female founder | 77 | 237.6 | 35.89 | 163.2 | 316.4 |
| | Male founder | 22 | 281.0 | 35.22 | 220.0 | 334.8 |
| | Female offspring | 118 | 234.2 | 37.27 | 169.1 | 388.3 |
| | Male offspring | 122 | 282.6 | 52.62 | 198.9 | 458.6 |
| $\dot{V}O_2$ max (ml $O_2$/hr)[e] | Female founder | 77 | 237.6 | 35.89 | 163.2 | 316.4 |
| | Male founder | 22 | 294.8 | 37.83 | 231.2 | 360.7 |
| | Female offspring | 118 | 247.5 | 38.78 | 180.2 | 397.7 |
| | Male offspring | 123 | 295.8 | 57.13 | 203.0 | 485.4 |
| Avg. body mass (g) from exercise $\dot{V}O_2$ trials[f] | Female founder | 77 | 22.28 | 2.122 | 17.52 | 27.67 |
| | Male founder | 22 | 27.65 | 2.787 | 23.41 | 32.62 |
| | Female offspring | 118 | 21.65 | 1.795 | 17.86 | 26.59 |
| | Male offspring | 122 | 26.92 | 2.910 | 19.31 | 34.80 |

[a] Food was removed from cages at about 1800 hr (CST) on the night prior to BMR determination; actual time was used as a covariate for analyses. Mean fast length (±SD) to start of BMR trial as 13.8 ± 0.68 (range 11.5–15.1) hours. Total fast length (±SD) to end of BMR trial was 23.8 ± 0.91 (range 20.6–25.7) hours.

[b] The term "founder" refers to measurements on breeder and nonbreeder males and females obtained from Harlan Sprague Dawley.

[c] Mean age (±SD) at BMR was 35.4 ± 2.57 (range 30–43) days.

[d] Reported exercise $\dot{V}O_2$ numbers were not corrected for washout characteristics of the metabolic chamber. Values for instantaneous corrected and steady-state $\dot{V}O_2$ values were similar (see text).

[e] One mouse died after the first trial.

[f] Mean age (±SD) at $\dot{V}O_2$max was 41.9 ± 2.84 (range 37–49) days.

## RESULTS

**Repeatability:** Levels of individual variation for whole-animal BMR and exercise $\dot{V}O_2$ were similar (Table 1; coefficients of variation, *CV*, of ~20%) and somewhat greater than for body mass (Table 1; *CV* 10–15%). The correlation between the lowest and second lowest hourly values of BMR within a day was 0.95 ($N = 365$). Individual differences in body mass and $\dot{V}O_2$ during treadmill exercise were also repeatable between trial days. Repeatabilities between trials were 0.84 for log instantaneous-corrected $\dot{V}O_2$max, 0.85 for log steady-state $\dot{V}O_2$max, and 0.98 for log body mass measured on the two trial days. (All correlations were significantly different from zero and none was significantly different from unity.) After accounting for the effects of statistically significant covariates, including body mass, the two exercise $\dot{V}O_2$ trials remained significantly correlated ($r = 0.53$), although this correlation was significantly less than unity.

Instantaneous $\dot{V}O_2$max averaged 4.0 ± 2.10% (±SD, min = 0.6, max = 9.5%, $N = 340$) higher than the corresponding steady-state $\dot{V}O_2$max values, and the two measures were highly correlated (day 1: $r = 0.99$; day 2: $r = 0.99$). However, we report results for the steady-state values only because the instantaneous-corrected $\dot{V}O_2$ data tended not to converge under REML (see below and APPENDIX). No difference in steady-state $\dot{V}O_2$max was found between trials 1 and 2 (mean = +1.13%, min = −31.7%, max = +39.9%; paired *t*-test = 1.822, d.f. = 338, $P = 0.069$). Body mass also did not differ significantly between trial days (mean = +0.2%, min = −8.7%, max = +9.3%; paired *t*-test = 1.12, d.f. = 338, $P = 0.265$).

**Removing effects of covariates before genetic analyses:** Body mass was highly phenotypically correlated with the metabolic traits, explaining 41% of the variation in BMR (Figure 1; Table 2) and about 50% of the variance for the measures of exercise $\dot{V}O_2$ (Figure 2; Table 2). Differences among measurement blocks also accounted for statistically significant amounts of variation for $\dot{V}O_2$max (~13%), but explained <2% of the variation in BMR (Table 2). Differences between parents (founders) and offspring accounted for a small but statistically significant proportion of variance for average exercise $\dot{V}O_2$ and body mass (Table 2). We did not find significant differences between parents and offspring for BMR or $\dot{V}O_2$max (Table 2). The multiple regressions did not

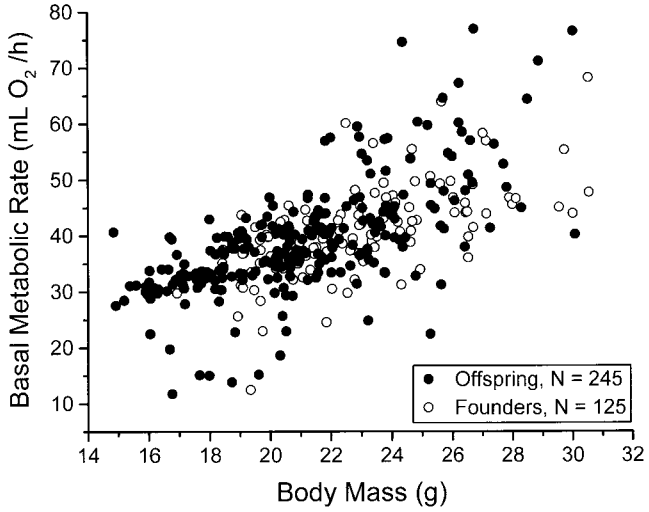


FIGURE 1.—Basal metabolic rates of 370 outbred Hsd:ICR house mice [open circles are founders (see text for definition), solid circles are offspring] in relation to body mass. The effects of other covariates (see Table 2) are not controlled.

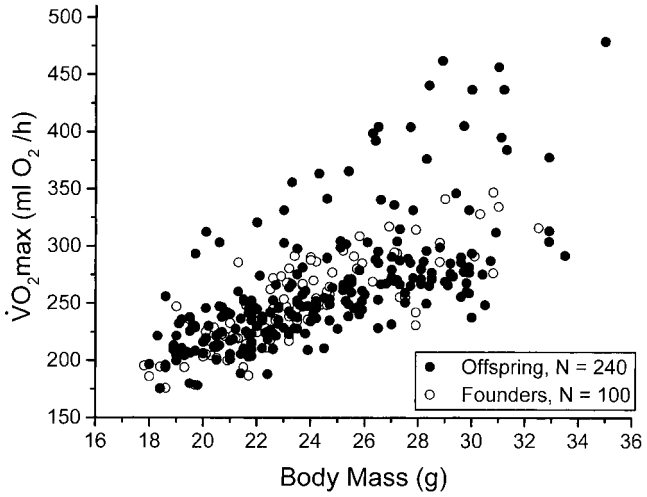


FIGURE 2.—Maximal oxygen consumption ($\dot{V}O_2$max) of 340 Hsd:ICR mice [open circles are founders (see text for definition), closed circles are offspring] in relation to body mass. The effects of other covariates (see Table 2) are not controlled.

indicate any significant differences attributable to sex, nor did we detect a significant sex-by-founder interaction.

**Genetic and environmental variance estimates:** Heritability estimates calculated from the univariate models are reported in Table 3. Variance components and standard errors are provided in the APPENDIX. Based on the *AE* models, the narrow-sense heritabilities were 0.09 for residual BMR (*AE vs. E*, $\chi^2 = 1.784$, $P > 0.10$), 0.57 for residual average $\log_{10}$ exercise $\dot{V}O_2$ (*AE vs. E*, $\chi^2 = 25.085$, $P < 0.001$), 0.64 for residual $\log_{10}$ $\dot{V}O_2$max (*i.e.*, the higher of the two trial measurements; *AE vs. E*, $\chi^2 = 23.127$, $P < 0.001$), 0.33 for $\log_{10}$ body mass during the BMR trials (*AE vs. E*, $\chi^2 = 19.093$, $P < 0.001$), and 0.42 for the average of the two body mass ($\log_{10}$) from the $\dot{V}O_2$ trials (*AE vs. E*, $\chi^2 = 23.579$, $P < 0.001$).

Results from three- (*ANE*, *ADE*, *ACE*) and four-(*ANCE* and *ADCE*) component models suggest statistically significant contribution of prenatal (or dominance genetic) effects variance for BMR (*e.g.*, *ANE vs. AE*, $\chi^2 = 11.712$, $P < 0.001$), average $\dot{V}O_2$ (*e.g.*, *ANE vs. AE*, $\chi^2 = 59.872$, $P < 0.001$), $\dot{V}O_2$max (*e.g.*, *ANE vs. AE*, $\chi^2 = 38.928$, $P < 0.001$), and for the measures of body mass (*e.g.*, *ANE vs. AE*, $\chi^2 = 8.974$, $P < 0.001$; Table 3; see APPENDIX for models with dominance effects). Postnatal environmental effects ($V_C$) under the four component models were generally negative for BMR and $\dot{V}O_2$max,

**TABLE 2**

**Statistically significant ($P \leq 0.05$) covariates from multiple regression equations for body mass and metabolic traits**

| Covariates | BMR | Steady-state avg. exercise $\dot{V}O_2$ | Steady-state $\dot{V}O_2$max | Body mass | |
|---|---|---|---|---|---|
| | | | | At start of BMR trial[a] | Avg. from $\dot{V}O_2$ trials |
| Body mass[a] | 41.2 | 53.4 | 52.1 | | |
| Sex[b] | | | | 23.0 | 52.7 |
| Founder | | | | 4.9 | 1.9 |
| Age at measurement[c] | 1.6 | 6.7 | 7.0 | 23.6 | 3.5 |
| Measurement block | 1.8 | 10.8 | 10.8 | 3.0 | 2.4 |
| Fasting time[c] | 2.4 | | | 1.1 | 1.1 |
| Multiple $R^2$ | 47.0 | 70.1 | 67.6 | 55.6 | 60.5 |

Values are squared partial correlation coefficients, in percentages. Residuals from the multiple regression equations were used for estimation of quantitative genetic parameters. See text for variable identification and coding.

[a] Body mass measured prior to placing mice into metabolic chambers.

[b] The sex-by-founder interaction term was not statistically different for any trait and, therefore, was omitted from the table.

[c] Includes both first- and second-order (*e.g.*, age squared) terms.

**TABLE 3**

**Standardized estimates of variance components from full and reduced univariate genetic models**

| | | *ANCE* | *ANE* | *ACE* | *AE* |
|---|---|---|---|---|---|
| BMR | $h^2$ | −0.11 | −0.11 | 0.08 | 0.09 |
| | $n^2$ | 0.28* | 0.23* | | |
| | $c^2$ | −0.12 | | 0.02 | |
| | $e^2$ | 0.95 | 0.88 | 0.90 | 0.91 |
| | *LL* | *−786.965* | *−788.871* | *−794.701* | *−794.727* |
| Steady state, avg. $\dot{V}O_2$ | $h^2$ | −0.35 | −0.34 | −0.13 | 0.57* |
| | $n^2$ | 0.70* | 0.64* | | |
| | $c^2$ | −0.09 | | 0.51* | |
| | $e^2$ | 0.74 | 0.70 | 0.62 | 0.43 |
| | *LL* | *−560.158* | *−561.850* | *−581.737* | *−591.786* |
| Steady state, $\dot{V}O_2$max | $h^2$ | −0.29 | −0.29 | −0.12 | 0.64* |
| | $n^2$ | 0.57* | 0.53* | | |
| | $c^2$ | −0.07 | | 0.44* | |
| | $e^2$ | 0.79 | 0.76 | 0.68 | 0.36 |
| | *LL* | *−585.074* | *−585.659* | *−600.574* | *−605.123* |
| Body mass at start of BMR trial | $h^2$ | 0.18 | 0.18 | 0.31* | 0.33* |
| | $n^2$ | 0.17* | 0.19* | | |
| | $c^2$ | 0.10 | | 0.16* | |
| | $e^2$ | 0.55 | 0.63 | 0.54 | 0.67 |
| | *LL* | *−392.929* | *−394.033* | *−396.183* | *−398.521* |
| Average body mass from two $\dot{V}O_2$ trials | $h^2$ | 0.26 | 0.26 | 0.38* | 0.42* |
| | $n^2$ | 0.17* | 0.19* | | |
| | $c^2$ | 0.10 | | 0.18* | |
| | $e^2$ | 0.47 | 0.55 | 0.44 | 0.58 |
| | *LL* | *−554.488* | *−555.443* | *−556.496* | *−559.110* |

Traits were residuals from multiple regression equations (see Table 2). Models tested included two full models, *ANCE* and *ADCE* (see APPENDIX); two models with three components, *ANE* and *ACE*; and a reduced model, *AE*, with only additive genetic and environmental variances (*i.e.*, all four variances tested), where *A* is the additive genetic variance, *N* is the prenatal maternal effects variance, *D* is the dominance genetic variance, *C* is the postnatal common environmental variance, and *E* is the environmental variance. Components as a proportion of the total phenotypic variance are: $h^2$, narrow-sense heritability; $n^2$, prenatal maternal effects; $c^2$, postnatal common environment variance; and $e^2$, environmental error variance. Tests of statistical significance of $h^2$ were assessed by constraining $V_A$ to zero and obtaining the log-likelihood (*LL*) of subsequent reduced models. Twice the difference in *LL* is distributed approximately as a chi square ($\chi^2$). Each test of the variance component is one-tailed with 1 d.f.; critical $\chi^2$ values are 2.706 at $P \leq 0.05$. *Statistically significant tests.

but positive for body mass (Table 3). For BMR, a test of the fit of *AE vs. ACE* confirmed no contribution of $V_C$ ($\chi^2 = 0.052$, $P > 0.50$), but a significant contribution of $V_C$ to average $\dot{V}O_2$ ($\chi^2 = 20.098$, $P < 0.001$) and $\dot{V}O_2$max ($\chi^2 = 9.098$, $P < 0.005$). We emphasize that the feasible estimates for additive genetic variance under the *AE* models may be biased and that alternative models that produce negative variance estimates (Table 3; APPENDIX) may lead to different conclusions from those we present (see DISCUSSION).

**Phenotypic, genetic, and environmental covariation:** The phenotypic correlation for whole-animal BMR and $\log_{10}$ $\dot{V}O_2$max (*i.e.*, not corrected for body mass or other covariates) was 0.43 ($N = 337$, $P < 0.001$). However, phenotypic correlations between residual BMR and measures of exercise $\dot{V}O_2$ were near zero ($r_P = \sim 0.05$, *e.g.*, Figure 3). The *AE* × *AE* reduced model indicated a positive genetic covariance between BMR and $\log_{10}$ $\dot{V}O_2$max residuals. A likelihood ratio test indicated that this genetic covariance was significantly different from zero ($\chi^2 = 5.747$, $P < 0.05$). The genetic correlation ($r_A$) between $\dot{V}O_2$max (steady state) and BMR residuals was 0.72. For comparison, the correlation from family ($N = 67$, dam only) means was 0.24 and also statistically different from zero ($P < 0.001$, Figure 3). The environmental covariance between BMR and $\log_{10}$ $\dot{V}O_2$max residuals was negative. As expected, both phenotypic and genetic correlations between the residual measures of body mass at the start of BMR and the average body mass from the two $\dot{V}O_2$max trials 1 wk later were positive and significantly different from zero ($r_P = 0.78$, but significantly less than 1; $r_A = 0.87$, no test because matrix became singular when additive covariance was dropped).

## DISCUSSION

**Implications for the aerobic capacity model:** The aerobic capacity model attempts to explain how the ener-

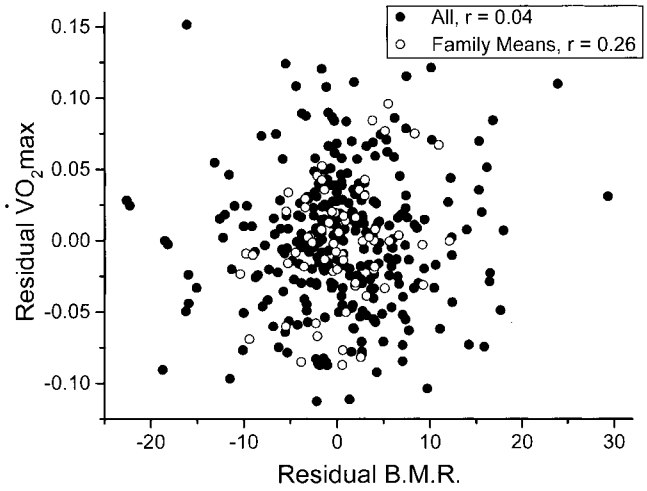


FIGURE 3.—Scattergram depicting absence of phenotypic correlation between $\dot{V}O_2$max residuals and BMR residuals ($r_P = 0.04$). Data points marked with open circles represent family (dam) means; the family mean correlation between $\dot{V}O_2$max and BMR residuals was 0.24 ($P < 0.001$).

getic costs incurred during the initial stages of the acquisition of endothermy might have been mitigated by the selective advantage resulting from greater ability to sustain aerobic locomotor activity (BENNETT and RUBEN 1979). The model postulates that BMR and $\dot{V}O_2$max are functionally linked, and a key implicit assumption is that in the ancestors of birds and mammals BMR and $\dot{V}O_2$max should have been positively genetically correlated. Although there are many reasons genetic correlations may not persist over evolutionary time, they may persist if the correlation reflects fundamental design features of the organism. If a linkage between BMR and $\dot{V}O_2$max is a fundamental design feature of terrestrial vertebrates in general, then extant terrestrial vertebrates should exhibit a positive genetic correlation. Hence, the presence of positive genetic correlations between BMR and $\dot{V}O_2$max in many species would lend support to the aerobic capacity model (see HAYES and GARLAND 1995 for a more detailed discussion).

Our results offer weak support for the aerobic capacity model. We detected a statistically significant, positive genetic correlation ($r_A = 0.72$) between residual BMR and residual $\log_{10}$ $\dot{V}O_2$max, but only under statistical models that assumed no contribution of either prenatal effects, dominance genetic effects, or common environmental effects to the phenotypic variance in either trait. Future studies will be required to determine whether such a correlation exists commonly in other animals and, by an appeal to parsimony, could be claimed as likely to have existed in the ancestors of mammals and/or birds. Nevertheless, the comparative approach of testing for the generality of a genetic correlation is a useful addition to the various tools, all of them indirect (see BENNETT 1991; HAYES and GARLAND 1995; RUBEN 1995), that have been used to address what is an inherently difficult problem, *i.e.*, inferring the details of an evolutionary shift in function that occurred at least 100 mya (see also GARLAND *et al.* 1997, 1999).

**Heritability:** Our findings of small additive genetic effects for BMR agree well with available estimates of $h^2$ of minimal or resting metabolic rates in other vertebrates (chickens, DAMME *et al.* 1986; humans, BOGARDUS *et al.* 1986; RICE *et al.* 1996). Average metabolic rates measured for up to 24 hr (SCHLESINGER and MORDKOFF 1963; SACHER and DUFFY 1979; MOODY *et al.* 1997, 1999; NIELSEN *et al.* 1997) and resting metabolism over 3 hr (KONARZEWSKI and DIAMOND 1995) differ among strains of mice, which suggests that genetic variance may be present for whole-animal metabolism in mice. Statistically significant among-litter variability for mass-specific resting metabolism was recently reported for armadillos (BAGATTO *et al.* 2000). However, these metabolic measures are not strictly comparable to the BMR measured by us. Lynch and colleagues (reviews in LYNCH 1992, 1994) reported low narrow-sense heritabilities (range 0.02–0.21) with substantial dominance variance for per-gram basal metabolism in another strain of genetically variable house mice (LACY and LYNCH 1979; LYNCH and SULZBACH 1984).

For residual $\dot{V}O_2$max, the reduced *AE* models indicated significant additive genetic variance (Table 3). Mass-corrected $\dot{V}O_2$max showed a significant, but small (6%), correlated response to selection for voluntary wheel-running behavior in this same strain of mice (SWALLOW *et al.* 1998b). This correlated selection response suggests that there is additive variance for $\dot{V}O_2$max in the Hsd:ICR strain. Studies of garter snakes also suggest broad-sense heritability for $\dot{V}O_2$max (GARLAND and BENNETT 1990). DOHM *et al.* (1994) found that hybrid female offspring of crosses between ICR and wild *M. domesticus* tended to resemble their wild progenitors for $\dot{V}O_2$max, suggesting dominance genetic effects. In humans, $h^2$ estimates of $\dot{V}O_2$max are generally low to moderate in magnitude (LESSAGE *et al.* 1985; BOUCHARD 1986; BOUCHARD *et al.* 1999, 2000).

Body mass was significantly heritable, as expected from previous quantitative genetic studies with this outbred strain of laboratory mice (*e.g.*, RISKA *et al.* 1984; DOHM *et al.* 1996). We also found small contributions from the common environmental component (Table 3), again in agreement with previous studies.

**How biased are *AE* models?** The breeding design we used permitted estimation of four components of variation: additive genetic, dominance genetic (or prenatal maternal effects), common environmental, and unique environmental variances. In a previous study (DOHM *et al.* 1996), we were generally able to estimate all four of these components for body mass, swimming endurance, and maximal sprint running speed measured on these same individual mice. Assuming prenatal effects rather than dominance genetic effects in the present study, the estimates for the full models im-

proved (*e.g.*, $V_E$ became positive), but estimates for $V_A$ remained negative. As noted above, our estimates of $V_A$ may be biased under the *AE* models. Bias can be of two kinds: either the estimate is quantitatively or qualitatively different from the true value. For BMR, the *AE* model fit nearly as well as did more complex models; standardized $V_A$ estimates were always around 10% (Table 3). However, for $\dot{V}O_2$max, $V_A$ estimates ranged from large and positive (0.64, *AE* model) to moderately large but negative (−0.29, *A[ND]CE* model; Table 3; appendix). If important variance components are omitted, then the residual errors are likely to be correlated (Lynch and Walsh 1998).

Did prenatal effects, common environmental effects, or dominance genetic variance contribute to variation in $\dot{V}O_2$max? In materials and methods, we noted that estimates of dominance genetic variance include prenatal shared environmental effects, if present. We therefore evaluated model fit assuming dominance (plus $V_A$, $V_C$, $V_E$) *vs.* the fit of a model with prenatal effects (again with $V_A$, $V_C$, $V_E$). The three- and four-component models indicated significant dominance or prenatal maternal effects, but because models with $V_D$ tended to yield negative estimates for environmental variance, we favored the fit of models with prenatal effects. Short of embryo transplant experiments (*e.g.*, Cowley 1991; Rhees *et al.* 1999) or more complex breeding designs than used here (*e.g.*, addition of maternal half-sibs), one cannot statistically separate the two components of variance in mammalian populations in the absence of an explicit assumption about the magnitude of dominance genetic effects. We believe that assumptions about the relative magnitudes of dominance genetic effects, or prenatal environmental effects, for BMR or $\dot{V}O_2$max are premature because virtually nothing is known about the genetic architecture of these traits. However, the effects of prenatal environment on BMR and $\dot{V}O_2$max under standard laboratory conditions were probably small. In support of this view, we note that Cowley (1991) found no prenatal effects on metabolically important organs (*e.g.*, mass of liver and kidney, brain size) in house mice. Finally, we found only minor contributions of common environmental effects on these metabolic traits, which suggests that maternal effects may not contribute significantly to individual variation in whole-animal metabolic traits in these mice (appendix; see also Dohm 1994).

Without $V_D$ or $V_N$ in the models, fit to the data was poor. If in fact the heritability of $\dot{V}O_2$max residuals is small in magnitude, what is the probability of obtaining negative $V_A$ given the breeding design employed by us? For $h^2$ of 1%, the probability of obtaining negative additive genetic variance from a half-sib data set of our size is greater than 50% (Lynch and Walsh 1998). However, because the animal model used in the REML procedure uses all of the information in the pedigree to yield variance estimates, the probability of obtaining negative variances was probably somewhat lower than for a comparable half-sib only design.

Variance components are positive by definition, but estimates of variance components in mixed linear models can be negative (Searle *et al.* 1992; Lynch and Walsh 1998). Negative variance components that account for only a few percent of total variance (*e.g.*, BMR, body mass) are best treated as zero, the result of sampling error (Lynch and Walsh 1998). However, negative variances accounted for a relatively large proportion (~50%) of the total phenotypic variance for $\dot{V}O_2$max when $V_D$ was included. However, when variances were estimated for models with prenatal effects rather than dominance genetic effects, the unique environmental variances were always positive. Negative variance estimates may also result from attempting to estimate too many causes of familial resemblance from sets of nonindependent groups of individuals. Our breeding design generated four sets of offspring resemblance, full- and half-sibs, with and without cross-fostering. If additive gene effects truly account for only small fractions of total phenotypic variance, then the component that contributes the majority to total variance may drive the fit. For example, when $V_D$ or $V_N$ was excluded from *ACE* and *AE* models for $\dot{V}O_2$max, estimates of $V_A$ were always positive and relatively large, suggesting that $h^2$ was overestimated and biased in these models. For models in which $V_D$ or $V_N$ was constrained to zero, part of the variance accounted for by dominance or prenatal effects was distributed among the other components, including $V_A$ (see also Shaw 1987; Wei and van der Werf 1993). The effect was most evident for $\dot{V}O_2$max, but was also evident, to a lesser extent, for body mass (Table 3; see also Dohm 1994; Dohm *et al.* 1996). Similar observations have been reported in the animal breeding literature (*e.g.*, Wei and van der Werf 1993; additional references in Dohm 1994), for results from simulation studies (Bridges and Knapp 1987; Shaw 1987), and for other traits measured on these same mice (Dohm *et al.* 1996).

Finally, negative variances may also result when an incorrect model is used. For example, failure to account for variance differences between sexes or between parents and offspring might inflate or minimize phenotypic differences among some groups of individuals in the pedigree (R. G. Shaw, personal communication). Although it is entirely possible that we may not have measured an influential factor, differences because of sex or parent and offspring effects cannot be part of the explanation for the large negative variance estimates obtained for $\dot{V}O_2$max. The variances did not differ between sexes for $\log_{10}$ $\dot{V}O_2$max or for the residuals upon which genetic analyses were conducted.

**Conclusions:** Despite our reporting of a significant, positive genetic correlation, we hasten to add that these results are tentative because the models are based on constraining dominance (or prenatal effects) and common environmental variance to zero. Without these con-

straints, we did not obtain theoretically viable parameter estimates for $\dot{V}O_2$max (*i.e.*, negative variance estimates were obtained). The constraints we imposed on the models may cause our estimates of additive variance to be biased if, in fact, these components contributed significantly to trait variation (Lynch and Walsh 1998). However, those other more general models sometimes appeared to fit the data better (with the significant exception of the problem of negative variance estimates) and also suggested that additive variance for both residual BMR and log $\dot{V}O_2$max was low or zero. Hence, the choice of models substantially affects the conclusions; alternative models and their interpretation are reported in Dohm (1994). Therefore, whereas we enthusiastically advocate the potential of the approach we have taken, we urge further study of the genetic covariance of metabolic traits in mice and indeed in other vertebrates that are amenable to quantitative genetic analyses.

This article was part of a dissertation presented by the first author to the University of Wisconsin in partial fulfillment of the requirements for the Ph.D. degree. The University of Wisconsin's animal care and use committee approved all protocols. R. G. Shaw and F. Shaw kindly provided a revised version of their maximum likelihood programs. We greatly appreciate R. Shaw's advice on model fitting and interpretation. We also thank C. Hannan and C. Kapke for their assistance with mouse husbandry and data entry and R. R. Peterson and staff for excellent animal care. P. A. Carter, M. R. Dentine, A. R. Ives, J. P. Hailman, W. H. Karasov, R. G. Shaw, and J. G. Swallow commented on and improved drafts of the manuscript. Financial support was provided by Karl Entemann and John Jefferson Davis fellowships (to M. R. Dohm), by a Michael Guyer postdoctoral fellowship (to J. P. Hayes), by grants from the National Science Foundation (IBN-9157268, Presidential Young Investigator Award; IBN-9111185; and IBN-9728434 to T. Garland, Jr.), and by the University of Wisconsin Graduate School.

## LITERATURE CITED

Astrand, P.-O., and K. Rodahl, 1986 *Textbook of Work Physiology: Physiological Bases of Exercise*. McGraw-Hill, New York.

Bagatto, B., D. A. Crossley II, and W. W. Burggren, 2000 Physiological variability in neonatal armadillo quadruplets: within- and between-litter differences. J. Exp. Biol. **203:** 1733–1740.

Bartholomew, G. A., D. Vleck and C. M. Vleck, 1981 Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. J. Exp. Biol. **90:** 17–32.

Bennett, A. F., 1991 The evolution of activity capacity. J. Exp. Biol. **160:** 1–23.

Bennett, A. F., and J. A. Ruben, 1979 Endothermy and activity in vertebrates. Science **206:** 649–654.

Blaxter, K., 1989 *Energy Metabolism in Animals and Man*. Cambridge University Press, Cambridge.

Bogardus, C., S. Lillioja, E. Ravussin, W. Abbott, J. K. Zawadzki *et al.*, 1986 Familial dependence of the resting metabolic rate. N. Engl. J. Med. **315:** 96–100.

Bouchard, C., 1986 Genetics of aerobic power and capacity, pp. 59–88 in *Sport and Human Genetics*, edited by R. M. Malina and C. Bouchard. Human Kinetics Publishers, Champaign, IL.

Bouchard, C., P. An, T. Rice, J. S. Skinner, J. H. Wilmore *et al.*, 1999 Familial aggregation of $\dot{V}O_2$max response to exercise training: results from the HERITAGE Family Study. J. Appl. Physiol. **87:** 1003–1008.

Bouchard, C., T. Rankinen, Y. C. Chagnon, T. Rice, L. Perusse *et al.*, 2000 Genomic scan for maximal oxygen uptake and its response to training in the HERITAGE family study. J. Appl. Physiol. **88:** 551–559.

Bridges, Jr., W. C., and S. J. Knapp, 1987 Probabilities of negative estimates of genetic variances. Theor. Appl. Genet. **74:** 269–274.

Carter, P. A., T. Garland, Jr., M. R. Dohm and J. P. Hayes, 1999 Genetic variation and correlations between genotype and locomotor physiology in outbred laboratory house mice (*Mus domesticus*). Comp. Biochem. Physiol. A **123:** 155–162.

Chappell, M. A., and L. R. G. Snyder, 1984 Biochemical and physiological correlates of deer mouse a-chain hemoglobin polymorphisms. Proc. Natl. Acad. Sci. USA **81:** 5484–5488.

Cheverud, J. M., 1982 Phenotypic, genetic, and environmental morphological integration in the cranium. Evolution **36:** 499–516.

Cowley, D. E., 1991 Genetic prenatal maternal effects on organ size in mice and their potential contribution to evolution. J. Evol. Biol. **4:** 363–382.

Damme, K., F. Pirchner, H. Willeke and H. Eichinger, 1986 Fasting metabolic rate in hens. 2. Strain differences and heritability estimates. Poultry Sci. **65:** 616–620.

Dohm, M. R., 1994 *Quantitative Genetics of Locomotor Performance and Physiology in House Mice* (*Mus domesticus*). University of Wisconsin, Madison, WI.

Dohm, M. R., C. S. Richardson and T. Garland, Jr., 1994 Exercise physiology of wild and random-bred laboratory house mice and their reciprocal hybrids. Am. J. Physiol. **267** (Regulatory, Integrative, and Comparative Physiology 36): R1098–R1108.

Dohm, M. R., J. P. Hayes and T. Garland, Jr., 1996 Quantitative genetics of sprint running speed and swimming endurance in laboratory house mice (*Mus domesticus*). Evolution **50:** 1688–1701.

Dutenhoffer, M. S., and D. L. Swanson, 1996 Relationship of basal to summit metabolic rate in passerine birds and the aerobic capacity model for the evolution of endothermy. Physiol. Zool. **69:** 1232–1254.

Falconer, D. S., and T. F. C. Mackay, 1996 *Introduction to Quantitative Genetics*, 4th ed. Longman Group, Essex, England.

Feder, M. E., and W. W. Burggren, (Editors), 1992 *Environmental Physiology of the Amphibians*. University of Chicago Press, Chicago.

Friedman, W. A., T. Garland, Jr. and M. R. Dohm, 1992 Individual variation in locomotor behavior and maximal oxygen consumption in mice. Physiol. Behav. **52:** 97–104.

Garland, T., Jr., and S. C. Adolph, 1991 Physiological differentiation of vertebrate populations. Annu. Rev. Ecol. Syst. **22:** 193–228.

Garland, T., Jr., and A. F. Bennett, 1990 Quantitative genetics of maximal oxygen consumption in a garter snake. Am. J. Physiol. **259** (Regulatory Integrative and Comparative Physiology 28): R986–R992.

Garland, T., Jr., and P. A. Carter, 1994 Evolutionary physiology. Annu. Rev. Physiol. **56:** 579–621.

Garland, T., Jr., T. T. Gleeson, B. A. Aronovitz, C. S. Richardson and M. R. Dohm, 1995 Maximal sprint speeds and muscle fiber composition of wild and laboratory house mice. Physiol. Behav. **58:** 869–876.

Garland, T., Jr., K. L. M. Martin and R. Diaz-Uriarte, 1997 Reconstructing ancestral trait values using squared-change parsimony: plasma osmolarity at the origin of amniotes, pp. 425–501 in *Amniote Origins: Completing the Transition to Land*, edited by S. S. Sumida and K. L. M. Martin. Academic Press, San Diego.

Garland, T., Jr., P. E. Midford and A. R. Ives, 1999 An introduction to phylogenetically based statistical methods, with a new method for confidence intervals on ancestral values. Am. Zool. **39:** 374–388.

Gatten, R. E., Jr., K. Miller and R. J. Full, 1992 Energetics at rest and during locomotion, pp. 314–377 in *Environmental Physiology of the Amphibians*, edited by M. E. Feder and W. W. Burggren. University of Chicago Press, Chicago.

Gromko, M. H., 1995 Unpredictability of correlated response to selection: pleiotropy and sampling interact. Evolution **49:** 685–693.

Hart, J. S., 1971 Rodents, pp. 2–149 in *Comparative Physiology of Temperature Regulation*, Vol. 2, edited by G. C. Whittow. Academic Press, New York.

Hauschka, T. S., and E. A. Mirand, 1973 The Breeder: "HA(ICR)" Swiss mouse, a multipurpose stock selected for fecundity, pp. 319–331 in *Perspectives in Cancer Research and Treatment*, edited by G. P. Murphy, D. Pressman and E. A. Mirand. Alan R. Liss, New York.

Hayes, J. P., 1989 Field and maximal metabolic rates of deer mice at low and high altitudes. Physiol. Zool. **62:** 732–744.

Hayes, J. P., and T. Garland, Jr., 1995 The evolution of endothermy: testing the aerobic capacity model. Evolution **49:** 836–847.

Hayes, J. P., and C. S. O'Connor, 1999 Natural selection on thermogenic capacity of high-altitude deer mice. Evolution **53:** 1280–1287.

Hayes, J. P., T. Garland, Jr. and M. R. Dohm, 1992 Metabolic rates and reproduction of *Mus*: are energetics and life history linked? Funct. Ecol. **6:** 5–14.

Hill, R. W., 1972 Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. J. Appl. Physiol. **33:** 261–263.

Hinds, D. S., R. V. Baudinette, R. E. MacMillen and E. A. Halpern, 1993 Maximum metabolism and the aerobic factorial scope of endotherms. J. Exp. Biol. **182:** 41–56.

Konarzewski, M., and J. Diamond, 1995 Evolution of basal metabolic rate and organ masses in laboratory mice. Evolution **49:** 1239–1248.

Lacy, R. C., and C. R. Lynch, 1979 Quantitative genetic analysis of temperature regulation in *Mus musculus*. I. Partitioning of variance. Genetics **91:** 743–753.

Lascoux, M., 1997 Unpredictability of correlated response to selection: linkage and initial frequency also matter. Evolution **51:** 1394–1400.

Lessage, R., J.-A. Simoneau, J. Jobin, J. Leblanc and C. Bouchard, 1985 Familial resemblance in maximal heart rate, blood lactate, and aerobic power. Hum. Hered. **35:** 182–189.

Lynch, C. B., 1992 Clinal variation in cold adaptation in *Mus domesticus*: verification of predictions from laboratory populations. Am. Nat. **139:** 1219–1236.

Lynch, C. B., 1994 Evolutionary inferences from genetic analyses of cold adaptation in laboratory and wild populations of the house mouse, *Mus domesticus*, pp. 278–301 in *Quantitative Genetic Studies of Behavior*, edited by C. R. B. Boake. University of Chicago Press, Chicago.

Lynch, C. B., and D. S. Sulzbach, 1984 Quantitative genetic analysis of temperature regulation in *Mus musculus*. II. Diallel analysis of individual traits. Evolution **38:** 527–540.

Lynch, M., and B. Walsh, 1998 *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Sunderland, MA.

Moody, D. E., D. Pomp and M. K. Nielsen, 1997 Variability in metabolic rate, feed intake and fatness among selection and inbred lines of mice. Genet. Res. **70:** 225–235.

Moody, D. E., D. Pomp, M. K. Nielsen and L. D. Van Vleck, 1999 Identification of quantitative trait loci influencing traits related to energy balance in selection and inbred lines of mice. Genetics **152:** 699–711.

Newman, S., J. J. Rutledge and B. Riska, 1989 Estimation of prenatal maternal genetic effects. J. Anim. Breed. Genet. **106:** 30–38.

Nielsen, M. K., L. D. Jones, B. A. Freking and J. A. DeShazer, 1997 Divergent selection for heat loss in mice. I. Selection applied and direct response through fifteen generations. J. Anim. Sci. **75:** 1461–1468.

Rhees, B. K., C. A. Ernst, C. H. Miao and W. R. Atchley, 1999 Uterine and postnatal maternal effects in mice selected for differential rate of early development. Genetics **153:** 905–917.

Rice, M. C., and S. J. O'Brien, 1980 Genetic variance of laboratory outbred Swiss mice. Nature **283:** 157–161.

Rice, T., A. Tremblay, O. Deriaz, L. Perusse, D. C. Rao *et al.*, 1996 Genetic pleiotropy for resting metabolic rate with fat-free mass and fat mass: the Quebec family study. Ob. Res. **4:** 125–131.

Richardson, C. R., M. R. Dohm and T. Garland, Jr., 1994 Metabolism and thermoregulation in crosses between wild and random-bred laboratory house mice *Mus domesticus*). Physiol. Zool. **67:** 944–975.

Riska, B., W. R. Atchley and J. J. Rutledge, 1984 A genetic analysis of targeted growth in mice. Genetics **107:** 79–101.

Roff, D., 1997 *Evolutionary Quantitative Genetics*. Chapman & Hall, New York.

Ruben, J., 1995 The evolution of endothermy in mammals and birds: from physiology to fossils. Annu. Rev. Physiol. **57:** 69–95.

Sacher, G. A., and P. H. Duffy, 1979 Genetic relation of life span to metabolic rate for inbred mouse strains and their hybrids. Fed. Proc. **38:** 184–188.

Schlesinger, K., and A. M. Mordkoff, 1963 Locomotor activity and oxygen consumption variability in two inbred strains of mice and their $F_1$ hybrids. J. Hered. **54:** 177–182.

Schmidt-Nielsen, K., 1984 *Scaling: Why Is Animal Size So Important?* Cambridge University Press, Cambridge.

Searle, S. R., G. Casella and C. E. McCulloch, 1992 *Variance Components*. John Wiley & Sons, New York.

Shaw, F. H., and C. J. Geyer, 1997 Estimation and testing in constrained covariance component models. Biometrika **84:** 95–102.

Shaw, R. G., 1987 Maximum likelihood approaches applied to quantitative genetics of natural populations. Evolution **41:** 812–826.

Swallow, J. G., P. A. Carter and T. Garland, Jr., 1998a Artificial selection for increased wheel-running behavior in house mice. Behav. Genet. **28:** 227–237.

Swallow, J. G., T. Garland, Jr., P. A. Carter, W.-Z. Zhan and G. C. Sieck, 1998b Effects of voluntary activity and genetic selection on aerobic capacity in house mice (*Mus domesticus*). J. Appl. Physiol. **84:** 69–76.

Turelli, M., 1988 Phenotypic evolution, constant covariances, and the maintenance of additive variance. Evolution **42:** 1342–1347.

Wagner, P. D., 1996 Determinants of maximal oxygen transport and utilization. Annu. Rev. Physiol. **58:** 21–50.

Walton, B. M., 1993 Physiology and phylogeny: the evolution of locomotor energetics in hylid frogs. Am. Nat. **141:** 26–50.

Wei, M., and J. H. J. van der Werf, 1993 Animal model estimation of additive and dominance variances in egg production traits of poultry. J. Anim. Sci. **71:** 57–65.

Weibel, E. R., C. R. Taylor, J.-M. Weber, R. Vock, T. J. Roberts *et al.*, 1998 Design of the oxygen and substrate pathways. VII. Different structural limits for oxygen and substrate supply to muscle mitochondria. J. Exp. Biol. **199:** 1699–1709.

Withers, P. C., 1977 Measurement of $\dot{V}O_2$, $\dot{V}CO_2$, and evaporative water loss with a flow-through mask. J. Appl. Physiol. **42:** 120–123.

Communicating editor: R. G. Shaw

## APPENDIX

The breeding design used in this study was not able to separate prenatal effects from dominance genetic variance. Therefore, data (residuals from multiple regressions) were analyzed in two ways: one assuming dominance (*ADCE*), the other (*e.g.*, *ANCE*) assuming only prenatal effects. The full *ADCE* and *ANCE* models each included four estimable variance components; $V_A$, additive genetic variance; $V_D$, dominance genetic (plus prenatal maternal, if present) variance; $V_N$, prenatal maternal effects (plus dominance genetic variance, if present); $V_C$, postnatal maternal effects and common environmental variance; $V_E$, environmental error variance; and NE refers to components that could not be estimated. For average exercise $\dot{V}O_2$ and $\dot{V}O_2$max (instantaneous only), the full *ADCE* model failed to converge. Therefore, estimates from the *ADE* and *ANE* models are reported. Variance components (±) standard errors of variance components (SE = [sampling variance]$^{0.5}$), and log-likelihood values (*LL*) for the full *ADCE* and *ANCE* models are presented.

**TABLE A1**

***ADCE:* Full model with dominance variance**

| Trait | $V_A$ | SE | $V_D$ | SE | $V_C$ | SE | $V_E$ | SE | *LL* |
|---|---|---|---|---|---|---|---|---|---|
| Basal metabolic rate | −5.181 | 2.6342 | 51.875 | 12.2103 | −5.782 | 1.8681 | 6.278 | 9.5532 | −786.9654 |
| Avg. exercise $\dot{V}O_2$, steady state | −6.467 | 0.8779 | 51.838 | 6.6908 | −1.727 | 0.4619 | −25.172 | 4.8248 | −560.1582 |
| Avg. exercise $\dot{V}O_2$, instantaneous | −6.924 | 0.7197 | 49.604 | 6.4368 | NE | NE | −24.568 | 4.6666 | −557.7498 |
| $\dot{V}O_2$max, steady state | −5.728 | 1.0626 | 44.731 | 6.8402 | −1.251 | 0.6875 | −18.140 | 4.9559 | −585.0741 |
| $\dot{V}O_2$max, instantaneous | −8.084 | 0.7424 | 54.135 | 6.6633 | NE | NE | −24.452 | 4.8345 | −594.2723 |
| Body mass at BMR trial | 0.793 | 0.3902 | 2.990 | 1.1108 | 0.426 | 0.2468 | 0.2021 | 0.8544 | −392.9290 |
| Avg. body mass from exercise $\dot{V}O_2$ trials | 4.013 | 1.5838 | 10.286 | 3.9584 | 1.522 | 0.8520 | −0.368 | 3.0074 | −554.4879 |

**TABLE A2**

***ANCE*: Full model with prenatal effects variance**

| Trait | $V_A$ | SE | $V_N$ | SE | $V_C$ | SE | $V_E$ | SE | *LL* |
|---|---|---|---|---|---|---|---|---|---|
| Basal metabolic rate | −5.181 | 2.6342 | 12.969 | 3.0526 | −5.782 | 1.8681 | 45.185 | 3.5290 | −786.9654 |
| Avg. exercise $\dot{V}O_2$, steady state | −6.467 | 0.8779 | 12.959 | 1.6727 | −1.727 | 0.4619 | 13.706 | 0.9564 | −560.1582 |
| Avg. exercise $\dot{V}O_2$, instantaneous | −6.924 | 0.7197 | 12.401 | 1.6092 | NE | NE | 12.636 | 0.7502 | −557.7498 |
| $\dot{V}O_2$max, steady state | −5.728 | 1.0626 | 11.183 | 1.7101 | −1.251 | 0.6875 | 15.408 | 1.1601 | −585.0741 |
| $\dot{V}O_2$max, instantaneous | −8.084 | 0.7424 | 13.534 | 1.6658 | NE | NE | 16.149 | 0.8519 | −594.2723 |
| Body mass at BMR trial | 0.793 | 0.3902 | 0.747 | 0.2777 | 0.426 | 0.2468 | 2.445 | 0.3093 | −392.9290 |
| Avg. body mass from exercise $\dot{V}O_2$ trials | 4.013 | 1.5838 | 2.572 | 0.9896 | 1.522 | 0.8520 | 7.347 | 1.1308 | −554.4879 |